

Studies in the Experimental Analysis of Sex.

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Part 5.—On the Effects of Testis-extract Injections upon Fowls.

IN the 'Proceedings of the Royal Society of Medicine,' vol. i, "Pathology," p. 153, 1907-8, Dr. C. E. Walker describes an experiment in which he injected two adult hens with extract of cock's testis for a certain period, with the apparent result that the combs grew very rapidly to quite twice their original area. On ceasing the injections they shrank gradually until they nearly reassumed their original size. A further experiment is alluded to in which several young hens of two months' age were treated in the same manner, and it is stated that, though the results differed somewhat from the first experiment, they were entirely satisfactory, but no further details, as far as I am aware, have been given.

On the strength of the above experiments Dr. Walker concludes, firstly, that the hen bird possesses the potentiality of developing the comb as in the male; secondly, that there is present some internal secretion in the testis which, when injected subcutaneously into the hen, calls forth the production of this and other secondary sexual characters proper to the male, e. g. the wattles and temperament.

This conclusion has been accepted, as proved by Dr. Walker's experiments, by a number of authorities on sex.

Since this conclusion, that the injection of testis extract into the female calls forth the production of certain secondary sexual characters of the male, is one of considerable theoretical importance, and since it rests at present entirely on the experimental evidence furnished by Dr. Walker, it appeared to me desirable to repeat the experiment on a rather larger scale, and to control the experiment with measurements on normal hens. It may be at once stated that the result of this inquiry has convinced me that the comb of the adult hen is usually in a state of fluctuating growth, varying between wide limits, and that this fluctuation is entirely uninfluenced by the injection of extracts of the cock's testis. Out of nine birds injected only one showed fluctuations in growth which fell outside the variability of the control birds, the other eight giving absolutely negative results. In the course of the experiments I also tested the influence of the injections upon the fertility of the eggs and upon the properties of the blood-serum of the injected birds.

The whole of the experiments have been done in the Department of Pathology, Oxford, under the supervision of Prof. Dreyer, to whom I tender my most hearty thanks for the help he has given me.

I. METHODS EMPLOYED.

As I was desirous of repeating Dr. Walker's experiments in the same manner as he performed them, the extract was made by crushing up the fresh testes of a cock with twice their weight of sterile saline and straining the emulsion through gauze. In this way everything except skin and connective tissue passes into the extract, which forms a fine emulsion. Dr. Walker injected the hens with 5 c.c. of such an emulsion every day. I have used various methods, in certain cases injecting the birds with greater amounts, up to as much as 10 c.c., at intervals of a few days, in other cases injecting

them every day with about 3 c.c. In all cases rather more extract was administered in my experiments in a given time than in Dr. Walker's. Since Dr. Walker obtained pronounced effects in three weeks, and very pronounced effects in less than two months, I have not continued the injections for more than a month except in a few cases. That this does not vitiate my results is, however, most clearly shown by the fact that in the two birds in which very marked variations in the comb were observed, comparable to Dr. Walker's, the full increase took place three weeks after the first injection, the injections being performed at intervals of two or three days. The injections were made with aseptic precautions in the pectoral muscles. In measuring the comb two methods have been used: firstly, by tracing an outline of the comb onto cardboard, and secondly, by measuring the two greatest dimensions of the comb directly with a pair of compasses. Both methods were used with the four birds in Experiment 1, but the numbers given in referring to this experiment in the schedule at the end were all taken from the tracings, as the direct measurements did not form a complete series for all the birds. As a consequence of this the numbers referring to comb measurement in these birds do not vary so smoothly as in the case of the later experiments. In Experiments 2 and 3, relating to fifteen birds, I relied entirely on direct measurements, which I consider liable to less experimental error. Measurements were not made of the wattles, as being too inconvenient. In calculating the percentage increase the following method is used. The height of the comb multiplied by the length, the same points being, of course, always taken, is considered as giving roughly the area of the comb. The increase of area observed is calculated as a percentage on the original area when the experiment began. Thus in Bird No. 1 the original area was 50×25 ; the area at the end of the injections was 72×37 , which gives, as the percentage increase—

$$\frac{(72 \times 37) - (50 \times 25)}{50 \times 25} \times 100 = 113 \text{ per cent.}$$

Besides measuring the combs the weights of the birds in grammes were taken at regular intervals, and as many observations as possible were made upon the fertility of the eggs by incubation. In certain cases samples of blood were drawn and their action upon suspensions of the testis extract was observed.

2. THE EFFECT ON THE GROWTH OF THE COMB.

In Experiment No. 1 four birds were used, three of them being white Leghorn hens of two years' age and one a buff Orpington of the same age. Two of the Leghorns were injected, namely, Nos. 1 and 2 in the schedule. No. 1 was injected with 35 c.c. extract in the course of twenty-four days. During this period the comb increased 113 per cent., the largest increase observed in any of the experiments. After the cessation of the injections the comb decreased a little and showed subsequent fluctuations of no very decided character, sometimes increasing considerably (see p. 11).

Bird No. 2 was injected with 102 c.c. in a period of seventy-five days. The comb fluctuated slightly in size, the greatest increase being 23 per cent.

In the two control birds, which were kept under the same conditions, but were not injected, one showed an increase of 24 per cent., the other remained constant.

The result of this experiment is that in one case the increase of comb in an injected bird was much greater than in the controls; the other injected bird, which was injected for a longer period, showed about the same increase as one of the control birds, and therefore gave a negative result. In the case of the injected bird which showed a large increase in the comb, there was no constant shrinking of the comb after the injections ceased.

The fluctuations in weight of all the birds did not show any correspondence with the fluctuations in the comb area.

In Experiment No. 2 six birds were used, all belonging to the same breed, viz. the Indian Jungle fowl, a small breed

a little larger than the ordinary Bantam. The combs in all these birds were similar single combs, but in Nos. 8, 9, and 10 the combs were larger, these birds being two years old and having a strain of Silky in them. The other three birds, Nos. 5, 6, and 7, were pure Jungle fowls of one year's age.

No. 5 was injected with 117 c.c. extract in sixty-two days. The increase of the comb was 76 per cent. After the cessation of the injections the comb fluctuated in size, but after having decreased a little it increased again, and five months after the injections had ceased it was rather larger in area than ever before. There was therefore no tendency to decrease after the cessation of the injections. Result doubtful, perhaps positive.

No. 6 was kept as a control bird from February 22nd to September 7th, during which period it showed an increase of comb of 29 per cent. It was then injected with 53 c.c. extract in twenty-one days, during which period the comb remained quite constant, showing no increase. Result entirely negative.

No. 7 was kept as a control from April 7th to June 14th, during which period the comb increased 16 per cent. It was then injected with 45 c.c. extract in fifteen days, during which it showed an increase of 35 per cent. The comb decreased again in August, but spontaneously increased 35 per cent. in September without any injections being administered, this increase being the same as that observed while the injections were going on. Subsequently, in November, the comb again increased, bringing up the percentage increase to 78. The result of injection was therefore entirely negative.

Bird No. 8 was injected with $71\frac{1}{2}$ c.c. extract in fifty-two days, during which period the comb increased 14 per cent. Result negative.

Nos. 9 and 10 were kept as controls during the whole period of the injections of the other birds, and they showed percentage increases of 33 and 31.

The result of this experiment, then, is that in no case was the percentage increase of the comb greater in the injected

bird during injection than in the control birds. The comb of the injected bird No. 5, which showed a large increase during injection, not only did not constantly shrink after the injections ceased, but actually, five months subsequently, attained its maximum size. Again, in these birds there was no correlation between growth of comb and general body-weight.

In Experiment No. 3 nine young birds, three months old, of the same parentage and brought up together, were used. They belonged to the Indian Jungle fowl breed, and all had similar combs. Three birds, Nos. 11, 12, and 13, were injected each with 39 c.c. extract in a period of twenty-one days, during which period their combs showed a percentage increase of 45, 62, and 30 respectively. The six control birds showed the following percentage increases during the same period: 53, 60, 62, 38, 14, and 30.

In case it might be argued that the effects of the injection might show themselves some time after the cessation of injection, measurements of the comb were continued for a month after the last injection. In that month the injected birds gave percentage increases of 26, 20, and 28, while the control birds in the same period gave 97, 9·8, 13, 35, 36, and 36.

The result of this experiment, therefore, conclusively showed that in young birds of three months old the injection of 39 c.c. of testis extract in a period of twenty-one days had absolutely no effect on the growth of the comb.

Summarising the results of the three experiments it will be seen that out of nine injected birds, eight gave absolutely negative results when compared with the controls. It cannot be objected to these negative results that the hens were not injected with enough extract or for a sufficiently long period, since all of them received as much, or in most cases more, and for an equal or longer period than the bird No. 1, which might be claimed as showing positive results. This bird showed an increase greater than in any of the controls (113 per cent.), but con-

sidering the wide limits of variation in uninjected birds, from 0 to 78 per cent., it is certain that no significance can be attached to this single case. Neither did the behaviour of this bird, subsequent to the cessation of the injections, lend any support to the idea that the injections were the cause of the increase. In Dr. Walker's two birds, after the injections ceased, the combs steadily shrank back nearly to their original dimensions. In my bird the comb shrank a little, but then afterwards increased again. Doubtless a larger series of measurements during the spring months on white Leghorn fowls would reveal as wide a range of fluctuation in untreated as in this injected bird. I have now under observation four Leghorn hens, whose combs have decreased to less than half their area during moulting, and doubtless they will again increase in the spring.¹ The measurements both on injected and control birds establish the fact that in adult as well as in young hens the comb is in a state of fluctuating growth, the fluctuations being often marked within a few days. If we attempt to correlate the variations in the comb with the variations of body-weight as given in the fourth column of the schedule, it will be seen that a simultaneous increase in the comb and in the body-weight is only to be observed in the young hens in Experiment 3, where such a correspondence would be naturally expected. It appears to me that an increase of comb is to be observed just before the hens begin laying.² Thus a reference to the fifth column in the schedule will show that the correspondence is marked, especially in Bird No. 7. It will be seen that the increase of comb is not confined to any particular period of the year, but may take place in autumn as well as in spring.

¹ This supposition has been confirmed, a normal Leghorn hen giving a percentage increase of 130 in twenty days. This is the greatest increase observed in any bird, normal or injected.

² In the next study evidence will be produced proving that the sudden increase of the comb is strictly correlated with egg-laying.

3. THE EFFECT ON THE BODY-WEIGHT, GENERAL HEALTH, ETC.

The series of weights of injected and non-injected hens shows that the injections do not have any constant effect on the weight of the body, even in the young animals used in Experiment 3. With regard to effect on general health it is true that one of the injected birds (No. 1) became unwell after the injections, appearing anæmic and with reduced temperature, and another (No. 8) died soon after the injections ceased, but seven of the injected birds showed no symptoms of any discomfort, laid well, and maintained their weight. It is therefore very probable that the two ill-haps were either purely coincidents or else due to accidental infection, and not to any poisonous action of the extract injected. Dr. Walker gives as the result of his injections that the hens became quarrelsome and attacked cocks that were put in with them. It may be mentioned that no such characteristics were developed by any out of my nine injected birds.

4. THE EFFECT ON THE FERTILITY OF THE EGGS.

Since the extract contains a large quantity of ripe and partially ripe spermatozoa, it was interesting to inquire if the injected hens might be rendered immune against the cock's spermatozoa, and if the presence of an immune substance in the body-fluids might render fertilisation impossible or abnormal. In order to test this, eggs laid by injected hens during the period of their injection were incubated to the third or fourth day together with eggs from control birds and the result noted. As will be seen from the schedule, four of the injected birds laid eggs during the full period of injection, and these eggs were in all cases fertile and normal in development. From some of these eggs healthy young were actually raised which showed no abnormalities. We may conclude, therefore, that the injection of the extract has no influence either on egg-laying or on the fertilisation

or development of the egg. It is a remarkable fact that one of the control birds (No. 3) laid a very large proportion of eggs which developed abnormally, abnormalities of a greater or less degree being observed in more than 50 per cent. of the eggs. Some of these abnormally developing eggs were simply abnormal in having the chick not sufficiently forward at the fourth day of incubation with the area vasculosa rather bloodless, but others showed actual structural abnormalities, in two cases double-headed or double-bodied monsters being produced, in others the back being twisted into a peculiar shape. I have recorded this fact, as it demonstrates very clearly that the production of these abnormalities must have been a fixed character in this hen and not an accidental occurrence.

5. THE EFFECT ON THE BLOOD.

The normal serum of the fowl has a very powerful agglutinating effect upon the live spermatozoa of the cock. If a small quantity of the spermatozoa from the vas deferens is mixed with a small quantity of normal blood-serum, it is observed that in a few moments the spermatozoa, instead of being dispersed through the fluid, are agglutinated in clumps and stringy masses. In the space of a quarter of an hour most of the spermatozoa will be found to be motionless, and on transferring them to saline they do not recover their mobility but are shown to be irrecoverably damaged. If we perform this experiment with a suspension of the testis extract used in our experiments, the same agglutinating effect is observed in test-tube experiments, but the agglutination does not proceed so rapidly even when the tubes are incubated at 37° C., because the suspension does not consist only of spermatozoa but very largely of fatty materials and cellular débris. The agglutinating effect of the serum obtained from an injected hen (No. 2) was tested twice against a suspension of spermatozoa and of testis extract and the result compared with samples of normal serum. No increased agglutinating power

was observed in the serum drawn from the injected bird, nor did this serum stop the motion of spermatozoa any quicker than in the case of normal birds. The various dilutions of the serums obtained from injected and non-injected birds also gave similar results.

It was therefore found in the limited experiments performed that no immunising process could be detected as the result of the injections, and this negative effect is in accordance with the observations on health, comb growth, and fertility, as affected by the injections.

SUMMARY OF RESULTS.

(1) The injection of testis extract into hens was found to have an entirely negative effect on the increase of the comb in eight out of nine adult and young hens when compared with the fluctuations in growth observed in control birds. In one case the injected hen showed an increase of comb slightly greater than any observed in the control birds,¹ but the comb in this bird did not show the constant shrinkage, after cessation of injections, observed in the cases cited by Dr. C. E. Walker, and regarded by him as an essential feature of the experiment.

(2) The injections had no observable effect on the health, body-weight, fertility, blood properties, or any other features, although very large quantities were administered over periods varying from fifteen to seventy-five days.

(3) The result of the experiments is to show that, although Dr. Walker's observations were doubtless correct, his conclusion that the increase of the comb was due to the testis extract injected was erroneous. There is, therefore, no evidence that the testis contains an internal secretion which, when injected into the female, can call forth the production of any of the male secondary sexual characters.

¹ Since the MS. was in proof, a control bird of two and half years age has given a percentage increase in comb of 130 in a period of twenty days, thus exceeding that of any of the injected birds (see note on p. 7).

EXPERIMENT No. 1.—No. of Bird, 1; White Leghorn,
2 years old.

Date.	Treatment.	Comb. measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Feb. 1	Injected 2 c.c. extract	50 × 25	—	—
" 3	" 2 " "	50 × 25	—	—
" 7	" 4 " "	50 × 25	—	—
" 10	" 4 " "	58 × 29	1520	—
" 12	" 3 " "	57 × 28	1420	—
" 15	" 5 " "	59 × 30	1380	—
" 17	—	59 × 30	1510	—
" 19	Injected 5 c.c. extract	67 × 34	1520	—
" 22	" 5 " "	67 × 34	1650	—
" 24	" 5 " "	72 × 37	1670	Increase of 113 %.
" 28	—	72 × 37	1500	From this date to March 7th the bird was very unwell, too weak to perch, and anæmic in appearance.
March 4	—	—	1320	Recovering.
" 7	—	67 × 34	1320	Recovered.
" 14	—	67 × 34	1380	—
" 18	—	67 × 34	1470	—
" 22	—	68 × 34	1510	—
" 24	—	—	—	Fertile normal egg.
" 25	—	70 × 36	1550	Increase of 10 % since March 7th.
" 30	—	70 × 36	1420	—
April 2	—	—	—	Fertile normal egg.
" 3	—	—	—	" "
" 4	—	—	—	" "
" 6	—	—	—	" "
" 7	—	70 × 34	1320	—
" 13	—	69 × 34	—	—
" 14	—	67 × 34	1080	—
" 19	—	67 × 34	1130	—
" 22	—	67 × 34	1200	—
" 26	—	66 × 33	1320	—
May 3	—	64 × 32	1410	—
" 14	—	59 × 30	1350	—
" 21	—	62 × 30	1240	—
June 1	—	61 × 28	1270	—
" 14	—	63 × 29	1290	—

No. of Bird, 2; White Leghorn, 2 years old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Feb. 1	Injected 2 c.c. extract	95 x 50	—	—
" 3	" 2 " "	—	—	—
" 7	" 4 " "	—	—	—
" 10	" 3 " "	95 x 50	2070	—
" 12	" 3 " "	95 x 50	1900	—
" 15	" 5 " "	97 x 52	1900	—
" 17	—	97 x 52	1850	—
" 19	Injected 5 c.c. extract	98 x 52	1970	—
" 24	" 5 " "	101 x 54	1900	—
March 3	—	98 x 53	1820	—
" 7	—	98 x 53	2050	—
" 11	Injected 5 c.c. extract	99 x 53	2020	—
" 14	" 5 " "	99 x 53	1870	Fertile normal egg.
" 17	" 10 " "	98 x 53	1860	—
" 22	" 5 " "	98 x 53	1700	—
" 25	" 10 " "	98 x 53	1680	—
" 30	" 10 " "	98 x 53	1629	Blood drawn for agglutination ex- periment.
April 6	" 10 " "	98 x 53	1650	Fertile normal egg.
" 12	—	—	—	" "
" 14	Injected 10 c.c. extract	98 x 53	1620	Normal fertile egg.
" 16	" 8 " "	98 x 53	1570	Increase of 23 %.
" 18	—	—	—	Blood drawn for agglutination ex- periment.
" 24	—	—	—	Normal fertile egg.
" 25	—	—	—	" — "
" 26	—	97 x 50	1790	—
May 1	—	—	—	Normal fertile egg.
" 3	—	95 x 50	1670	—
" 7	—	—	—	Normal fertile egg.
" 14	—	94 x 50	1560	" — "
" 21	—	94 x 50	1290	—
June 1	—	92 x 48	1320	—
" 14	—	92 x 48	1300	—

EXPERIMENT No. 2.—No. of Bird, 5; Jungle Fowl, 1
year old.

Date.	Treatment	Comb measure- ment in millimetres	Weight in grammes	Remarks.
1910				
Feb. 19	Injected 2 c.c. extract	23.5 × 10.25	720	
" 22	" 3½ " "	23.5 × 10.25	720	—
" 24	" 4 " "	23.5 × 10.5	740	—
" 28	" 5 " "	24 × 10.5	750	—
March 3	" 5 " "	25 × 10.25	780	—
" 5	" 5 " "	26 × 10.5	760	—
" 8	" 3½ " "	27 × 11	800	—
" 11	" 5 " "	27.5 × 11.5	800	—
" 14	" 5 " "	28 × 11	820	—
" 17	" 9 " "	28.5 × 12	800	—
" 22	" 5 " "	29 × 12	800	—
" 25	—	29 × 12	760	—
" 28	Injected 10 c.c. extract	—	—	Normal fertile egg.
" 30	" 10 " "	29 × 12	720	" "
April 6	" 10 " "	29 × 12	740	—
" 7	—	—	—	Normal fertile egg.
" 8	—	30 × 11½	690	—
" 12	—	—	—	Normal fertile egg.
" 14	Injected 10 c.c. extract	30 × 12	740	—
" 16	" 10 " "	30 × 12	740	Normal fertile egg.
" 19	" 10 " "	30 × 12.75	710	—
" 22	" 5 " "	30.5 × 12.5	770	Increase of 76 %.
" 26	—	31.5 × 13.5	840	—
May 3	—	32 × 13	810	—
" 10	—	31.25 × 13	720	—
" 17	—	31 × 13	650	—
" 24	—	29 × 12	630	—
" 31	—	29 × 12	620	—
June 6	—	30 × 12.5	670	—
" 14	—	30 × 12.5	670	—
" 20	—	30 × 12	670	—
" 24	—	31 × 13	680	—
July 1	—	30.5 × 12.75	690	—
" 7	—	31 × 12.25	700	—
" 9	—	31 × 12	710	—
" 14	—	31 × 12	720	—
" 22	—	33 × 14.5	740	Largest dimen- sions of comb reached.
Aug. 16	—	30 × 12.5	770	—
Sept. 9	—	29 × 12	700	—
" 10	—	28 × 12	720	—
" 14	—	29 × 12	770	—
" 16	—	30 × 12.5	800	—
" 19	—	30 × 12	720	—
" 23	—	29 × 12	790	Egg laid.
" 28	—	30 × 12.5	820	—
" 30	—	31 × 12	840	Egg laid.
Oct. 3	—	32 × 13	840	—

No. of Bird, 6; Jungle Fowl, 1 year old.

Date.	Treatment.	Comb measure- in millimetres.	Weight in grammes	Remarks.
1910				
Feb. 22	—	22 × 7	670	—
March 1	—	22 × 7.5	660	—
" 8	—	22 × 7	720	—
" 18	—	23 × 7.5	770	—
" 22	—	23.5 × 7.25	690	—
" 25	—	23.5 × 7.5	720	—
" 30	—	23.5 × 7.5	700	—
April 6	—	22.5 × 7.25	700	—
" 14	—	23 × 7.5	640	Several normal fertile eggs.
" 19	—	23.5 × 8	640	
" 22	—	23.5 × 8	690	
" 26	—	23.5 × 8	660	
May 3	—	23.5 × 8	720	
" 10	—	24 × 8	670	
" 17	—	25 × 8	670	
" 24	—	24.5 × 7.5	600	—
" 31	—	24.5 × 7.25	650	—
June 7	—	24.25 × 7	660	—
" 14	—	24 × 7	600	—
" 20	—	24 × 7	630	—
" 24	—	24 × 7	650	—
July 1	—	24.5 × 7	690	—
" 7	—	24.5 × 7.25	670	—
" 9	—	25 × 7.25	690	—
" 14	—	24.5 × 7	660	—
" 22	—	24.5 × 7	630	—
Aug. 16	—	24 × 7	700	—
Sept. 7	—	24 × 7	690	Greatest increase, 29 %.
" 10	Injected 4 c.c. extract	24 × 7	690	—
" 12	" 5 " "	24 × 7	670	—
" 14	" 4 " "	24 × 7	670	—
" 16	" 4 " "	24 × 7	670	—
" 17	" 4 " "	—	—	—
" 19	" 4 " "	24 × 7	670	—
" 21	" 4 " "	24 × 7	640	—
" 23	" 4 " "	24 × 7	650	—
" 24	" 4 " "	—	—	—
" 26	" 4 " "	24 × 7	670	—
" 28	" 4 " "	24 × 7	670	—
" 30	" 4 " "	23.75 × 6.5	670	—
Oct. 1	" 4 " "	—	670	—
" 3	—	24 × 6.5	690	No increase as result of injection, slight decrease.

No. of Bird, 7; Jungle Fowl, 1 year old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes	Remarks.
1910				
April 7	—	26.25 × 9.25	840	
" 14	—	27 × 9.75	840	
" 19	—	27 × 10	820	
" 22	—	27 × 10	850	
" 26	—	26.5 × 10.25	870	
May 3	—	27 × 10.5	820	
" 10	—	26.5 × 10.25	820	
" 17	—	27 × 10.25	710	Several normal fertile eggs.
" 24	—	26.5 × 10	670	
" 31	—	26.5 × 10.25	670	
June 7	—	26 × 10	730	
" 14	—	27 × 10	770	Increase 16 %.
" 15	Injected 3 c.c. extract	—	740	
" 16	" 3 " "	27 × 10	770	
" 17	" 3 " "	27.25 × 10	720	
" 18	" 3 " "	28 × 11	750	
" 19	" 3 " "	28 × 11	740	
" 20	" 3 " "	28.5 × 11.5	740	—
" 21	" 3 " "	28.5 × 11	720	—
" 22	" 3 " "	28 × 11.5	720	—
" 23	" 3 " "	28.25 × 12	740	—
" 24	" 3 " "	28.5 × 12	710	Egg laid, normal.
" 25	" 3 " "	28.5 × 12	700	—
" 27	" 3 " "	28.5 × 12	700	Egg laid, normal.
" 28	" 3 " "	28.5 × 12	720	—
" 29	" 3 " "	29 × 12	710	Egg laid, normal.
" 30	" 3 " "	29.5 × 12.5	750	Increase of 36 %.
July 1	—	29.75 × 12	720	—
" 7	—	29.25 × 11	670	—
" 9	—	29 × 11	690	—
" 14	—	28 × 10.5	660	—
" 22	—	28 × 10.5	650	—
Aug. 16	—	27.5 × 10	750	—
Sept. 7	—	28 × 11	700	—
" 10	—	28.5 × 10.75	750	—
" 14	—	29.5 × 11.5	790	—
" 16	—	30 × 12	800	Egg laid.
" 19	—	29.5 × 11.5	750	Increase of 35 %.
" 21	—	30.5 × 12.25	850	since August 16th. Egg laid.
" 23	—	29.5 × 11.5	820	—
" 26	—	29.5 × 11.5	820	—
" 28	—	29.5 × 11.5	820	—
" 30	—	29.5 × 11	840	—
Oct. 3	—	29.5 × 11.25	840	—
" 18	—	31.75 × 12	890	Eggs laid.
" 25	—	34 × 13	900	—
Nov. 1	—	35 × 14	850	Increase of 78 %.
" 8	—	31.5 × 13	800	since August 16th.

No. of Bird, 8; Jungle Fowl, 2 years old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
March 1	Injected $3\frac{1}{2}$ c.c. extract	26.5 × 12	800	—
" 11	" 5 " "	27 × 12	790	—
" 14	" 5 " "	27 × 12	740	—
" 17	" 5 " "	27 × 12	730	—
" 22	" 5 " "	26.5 × 12	730	—
" 25	" 8 " "	26.5 × 12	750	—
" 30	" 8 " "	27.5 × 12.25	740	Normal fertile egg.
April 6	" 8 " "	27 × 12	770	Normal fertile egg.
" 8	—	27.25 × 12	750	—
" 14	Injected 5 c.c. extract	27 × 12.25	790	—
" 19	" 9 " "	27.5 × 12.5	820	—
" 22	" 10 " "	28 × 13	820	Increase of 14%.
" 26	—	27.5 × 12.75	800	—
" 30	—	—	—	Found dead on nest. Peritoneal cavity full of clear yellow pus. Slight infection of tuberculosis in liver. hardly sufficient as cause of death.

No. of Bird, 9; Jungle Fowl, 2 years old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
March 1	—	37.5 × 15	900	—
" 11	—	36 × 14	900	—
" 18	—	35.5 × 14	870	—
" 22	—	34.5 × 13.5	870	—
" 25	—	35 × 14	890	—
" 30	—	35 × 13.5	920	Several normal fertile eggs.
April 7	—	35 × 13.5	920	
" 14	—	35 × 14	950	
" 19	—	35 × 13.75	950	
" 22	—	36 × 14	970	
" 26	—	36 × 14	950	
May 5	—	37.15 × 14	990	
" 10	—	37.5 × 15	940	—
" 17	—	38 × 15	970	
" 24	—	39 × 15.25	990	
" 31	—	39 × 16	990	
June 5	—	39 × 16	1000	Increase of 33%.
" 14	—	38 × 15	950	Suffering from hard tumour-like swelling in abdomen. Killed, and on dissection oviduct found occluded with large masses of yolk; ovary also full of large-yolked eggs.

No. of Bird, 10; Jungle Fowl, 2 years old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight.	Remarks.
1910				
Feb. 23	—	20.5 × 12.75	—	Numerous fertile normal eggs laid.
March 3	—	20.5 × 12.75	—	
" 30	—	21 × 13.5	—	
April 25	—	23 × 15	—	
June 2	—	22 × 13	—	
" 24	—	20 × 13	—	Increase of 31%.
Sept. 26	—	23.5 × 13.5	—	

EXPERIMENT No. 3.—No. of Bird, 11; Jungle Fowl, 3 months old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	20 × 6.5	350	—
" 9	Injected 3 c.c. extract	20 × 6.75	350	—
" 12	" 3 " "	20.25 × 6.75	410	—
" 14	" 3 " "	20 × 6.75	420	—
" 16	" 3 " "	20 × 6.75	450	—
" 17	" 3 " "	—	—	—
" 19	" 3 " "	20 × 7.25	460	—
" 21	" 3 " "	20.25 × 7.25	500	—
" 23	" 3 " "	20.5 × 7.5	490	—
" 24	" 3 " "	—	—	—
" 26	" 3 " "	21 × 8	490	—
" 28	" 3 " "	21.25 × 8	500	—
" 30	" 3 " "	21.25 × 8	500	—
Oct. 1	" 3 " "	—	500	—
" 3	—	21.75 × 9	510	Increase of 45 %.
" 18	—	23.25 × 10	570	—
" 25	—	23.75 × 10	600	—
Nov. 1	—	24.5 × 10	600	—
" 8	—	24.75 × 10	600	Increase of 26 % since October 3rd.

No. of Bird, 12; Jungle Fowl, 3 months old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	18 × 5	350	—
" 9	Injected 3 c.c. extract	18.5 × 5.25	350	—
" 12	" 3 " "	19 × 5.75	420	—
" 14	" 3 " "	19 × 6	410	—
" 16	" 3 " "	19 × 6	450	—
" 17	" 3 " "	—	—	—
" 19	" 3 " "	19.5 × 6	470	—
" 21	" 3 " "	19.5 × 6	500	—
" 23	" 3 " "	19.75 × 6.5	500	—
" 24	" 3 " "	—	—	—
" 26	" 3 " "	20 × 6.5	500	—
" 28	" 3 " "	20 × 7	500	—
" 30	" 3 " "	20.5 × 7.25	520	—
Oct. 1	" 3 " "	—	520	—
" 3	—	21 × 7.5	520	Increase of 62
" 18	—	22.5 × 8	640	—
" 25	—	23 × 7.5	620	—
Nov. 1	—	23.25 × 8	600	—
" 8	—	23.75 × 8	600	Increase of 20 % since October 3rd.

No. of Bird, 13; Jungle Fowl, 3 months old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	16.5 × 4.75	270	—
" 9	Injected 3 c.c. extract	16 × 4.75	270	—
" 12	" 3 " "	16.25 × 4.75	340	—
" 14	" 3 " "	16.5 × 4.75	340	—
" 16	" 3 " "	16.75 × 5	380	—
" 17	" 3 " "	—	—	—
" 19	" 3 " "	16.75 × 5	400	—
" 21	" 3 " "	17 × 5	440	—
" 23	" 3 " "	16.75 × 5	440	—
" 24	" 3 " "	—	—	—
" 26	" 3 " "	17.25 × 5	430	—
" 28	" 3 " "	17.25 × 5.25	420	—
" 30	" 3 " "	17.5 × 5.5	440	—
Oct. 1	" 3 " "	—	440	—
" 3	—	18 × 5.5	450	Increase of 30 %.
" 18	—	19 × 6	550	—
" 25	—	19 × 6	570	—
Nov. 1	—	19.5 × 6.75	620	—
" 8	—	19.5 × 6.5	600	Increase of 28 % since October 3rd.

No. of Bird, 14; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	18.5 × 6	410	—
" 9	—	18.5 × 6	420	—
" 12	—	19 × 6	500	—
" 14	—	18.75 × 6	490	—
" 16	—	19 × 6.5	500	—
" 19	—	19.25 × 6.75	530	—
" 21	—	19.5 × 7	570	—
" 23	—	19.75 × 7	570	—
" 26	—	20 × 7	550	—
" 28	—	20.5 × 7	540	—
" 30	—	21 × 7.5	550	—
Oct. 3	—	21.25 × 8	540	Increase of 53%.
" 18	—	23 × 8.5	600	—
" 25	—	25.5 × 10	630	—
Nov. 1	—	27 × 11.25	650	—
" 8	—	28 × 12	650	Increase of 97% since October 3rd.

No. of Bird, 15; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	17 × 4	300	—
" 9	—	17 × 4	310	—
" 12	—	17.5 × 4.25	320	—
" 14	—	17 × 4.25	320	—
" 16	—	17.25 × 5	350	—
" 19	—	17.25 × 5	370	—
" 21	—	17.25 × 4.75	350	—
" 23	—	17.25 × 5	370	—
" 26	—	18 × 5	380	—
" 28	—	18.25 × 5.25	400	—
" 30	—	18.25 × 5.25	400	—
Oct. 3	—	19 × 5.75	410	Increase of 60%.
" 18	—	20 × 6.5	480	—
" 25	—	20 × 6.5	500	—
Nov. 1	—	21 × 7	500	—
" 8	—	20 × 6	570	Increase of 98% since October 3rd.

STUDIES IN THE EXPERIMENTAL ANALYSIS OF SEX. 611

No. of Bird, 16; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	15.5 × 3	250	—
" 9	—	15.75 × 3.5	260	—
" 12	—	15.75 × 3.5	300	—
" 14	—	16 × 3.5	300	—
" 16	—	15.75 × 3.5	320	—
" 19	—	15.5 × 3.75	320	—
" 21	—	15.5 × 4	350	—
" 23	—	16 × 4	350	—
" 28	—	16.25 × 4.25	370	—
" 30	—	16.5 × 4.25	370	—
Oct. 3	—	16.75 × 4.5	390	Increase of 62%.
" 18	—	17.5 × 5	490	—
" 25	—	17.75 × 4.75	500	—
Nov. 1	—	18 × 4.75	500	—
" 8	—	18 × 4.75	500	Increase of 13% since October 3rd.

No. of Bird, 17; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	16 × 4	250	—
" 9	—	16.5 × 4.25	270	—
" 12	—	16.25 × 4.25	310	—
" 14	—	16 × 4.5	320	—
" 16	—	16.5 × 4.75	340	—
" 19	—	16 × 4.75	380	—
" 21	—	16.5 × 4.75	370	—
" 23	—	16.75 × 4.75	380	—
" 26	—	17.25 × 5	380	—
" 28	—	17 × 5	390	—
" 30	—	17 × 5	400	—
Oct. 3	—	17.75 × 5	410	Increase of 38%.
" 18	—	19 × 5.75	500	—
" 25	—	19.25 × 5.75	510	—
Nov. 1	—	20 × 5.25	500	—
" 8	—	20 × 6	500	Increase of 35% since October 3rd.

No. of Bird, 18; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	17 × 4.5	300	—
" 9	—	17.5 × 4.5	350	—
" 12	—	17.5 × 4.5	370	—
" 14	—	18 × 4.25	370	—
" 16	—	18 × 4.25	390	—
" 19	—	17.5 × 4.25	420	—
" 21	—	17.5 × 4	430	—
" 23	—	17.75 × 4.5	450	—
" 26	—	17.75 × 4.5	460	—
" 28	—	18 × 4.75	470	—
" 30	—	18.25 × 4.75	470	—
Oct. 3	—	18.5 × 4.75	470	Increase of 14 ² / ₁₀ .
" 18	—	19 × 5.75	580	—
" 25	—	20 × 5.75	580	—
Nov. 1	—	20 × 6	570	—
" 8	—	20 × 6	570	Increase of 36 ² / ₁₀ since October 3rd.

No. of Bird, 19; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	17 × 5	280	—
" 9	—	17.25 × 5	280	—
" 12	—	17.5 × 5	320	—
" 14	—	17.25 × 5.25	320	—
" 16	—	17.25 × 5	340	—
" 19	—	17.5 × 5.5	370	—
" 21	—	17.5 × 5.5	380	—
" 23	—	18 × 5	390	—
" 26	—	18 × 5.75	400	—
" 28	—	18 × 6	400	—
" 30	—	18 × 6	410	—
Oct. 3	—	18.5 × 6	410	Increase of 30 ⁰ / ₁₀ .
" 18	—	19.5 × 6.25	510	—
" 25	—	19.5 × 6.75	550	—
Nov. 1	—	20 × 6.75	550	—
" 8	—	21 × 7.25	550	Increase of 36 ⁰ / ₁₀ since October 3rd.